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Genotype/Phenotype Correlations in Wilms' Tumor

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Study of genotype/phenotype relationships involving the Wilms' tumor (WT) gene, *WT1*, in WT patients has provided insights into the function of the *WT1* protein, a transcriptional regulator, and has suggested possible mutational mechanisms important in the etiology of WT. For example, the identification of deletion/insertion mutations in the first exon implicates a deletion hotspot consensus sequence in the etiology of these mutations. The disproportionate number of WT/aniridia patients with such mutations further suggest that this genetic mechanism may be enhanced by the hemizygous state. *WT1* mutations are observed throughout the gene and, as predicted by the two-hit mutational model, germline mutations predominantly occur in patients with congenital genitourinary (GU) anomalies and/or bilateral disease. The presence of hemizygous mutations in tumors from individuals with germline 11p13 deletions encompassing *WT1* supports the hypothesis that inactivation of both *WT1* alleles is important in tumorigenesis.

Analyses of *WT1* mutations in individuals with WT-associated Drash syndrome and WT patients with GU anomalies in the absence of Drash syndrome indicate that Drash patients almost invariably carry germline missense mutations in the zinc finger domains whereas WT/GU patients carry germline mutations that delete the *WT1* gene or encode truncated proteins. These data suggest a functional difference between mutant *WT1* protein carrying a single amino acid substitution versus mutant *WT1* protein that is grossly truncated or *WT1* haploinsufficiency.

These and other genotype/phenotype correlations in WT patients will be discussed in more detail.

Key words: genetic variants, WAGR syndrome, Wilms' tumor

INTRODUCTION

The study of genotype/phenotype correlations is the basis of a genetic approach to biological questions. Examination of the phenotypes associated with both normal and mutant genes can provide invaluable insights into the function of the protein products of the genes, the delineation of protein functional domains, and, in some cases, the mutational mechanism responsible for genetic variants. In the case of Wilms' tumor (WT), the recognition almost two decades ago that WAGR (WT/aniridia/genitourinary anomalies/mental retardation) syndrome patients—a particular phenotype—had a characteristic genotype—karyotypically identifiable constitutional 11p13 deletions—resulted in the localization of a WT gene, *WT1*, to that genomic region [1]. Further localization of the WT gene was dependent on mapping the breakpoints of the chromosome deletions carried by children with one or more of the WAGR phenotypes. Additionally, this type of analysis resulted in the delineation of WT and aniridia (AN) as two distinct loci [2]. In contrast, the inability to localize the WT and genitourinary (GU) phenotypes separately led to the hypothesis, later confirmed once the gene was isolated, that alteration of a single gene was responsible for both phenotypes [3].

Although the WAGR-associated germline 11p13 deletions were of major importance for the localization and subsequent isolation of a WT gene, WAGR patients account for only ~1% of all WT cases [4]. The observation of other, non-11p, constitutional karyotypic abnormalities in WT patients suggests genes at other genomic regions play a role in tumorigenesis. Non-11p germline karyotypic abnormalities such as trisomy 18, X monosomy (Turner syndrome), and 2q alterations have been reported in WT patients, albeit rarely [5–7]. In addition to WT, multiple developmental anomalies were exhibited in these individuals, which suggest that a generalized perturbation of development predisposes to WT. This hypothesis is supported by the rare, but notable, observation of individuals with WT in several families with developmental anomalies and/or an increased cancer risk [8–11].

Data from cytogenetic analyses of WT have indicated that roughly 10–30% of tumors display tumor-specific deletion of 11p [12–17]. In addition, non-11p numeric or structural abnormalities involving chromosomes 1, 6, 7, 8, 11, 12, 16, 17, and 18 have been observed nonrandomly [12,13,15–17]. Interestingly, as noted above, some of these somatic changes—11p duplication, 11q deletion,

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and trisomy 18—have been observed as germline aberrations in WT patients, again suggesting that genes in these regions play a role in tumorigenesis. Somatic alteration of 16, observed either as 16q deletions and/or 16q loss of heterozygosity (LOH), is observed in ~20% of tumors [12,15,17]. The presence of 16q LOH [18] has recently been correlated with a poor prognostic phenotype [19], consistent with the hypothesis that a locus at 16q is important in malignant progression [17,19].

The presence of chromosome 17 structural anomalies in ~15% of tumors and the observation of an individual with WT in a Li-Fraumeni family [8,17] suggested a role for the p53 gene, frequently mutated in a wide variety of human cancers, in WT development. Although p53 mutations were observed infrequently in tumors displaying a favorable histologic phenotype [20,21], p53 mutations were detected in 3 of 11 tumors displaying an anaplastic phenotype [22], which suggests a role for p53 in the development of a specific subset of WT.

As the only isolated WT gene to date, the *WT1* gene at chromosomal band 11p13 has been the focus of a wide variety of studies to investigate its function, its role in embryonic development, and its role in the development of WT and other malignancies. The *WT1* gene, consisting of 10 exons, encodes a transcription factor containing four Cys-His zinc finger domains encoded by exons 7-10 [23,24]. In vitro studies have suggested that the WT1 protein functions primarily as a transcriptional repressor, a role that requires the carboxy-terminal zinc finger domains for binding to target DNA sequences and less well-defined activation and repressor domains in the more amino-terminal portion of the protein [25–27]. Analyses of *WT1* mutations in patients have provided a number of insights into the role of this transcription factor in vivo and have suggested possible genetic mechanisms important in the alteration of this locus.

The first exon of *WT1* is greater than 70% of GC-rich and contains many short tandem di- and trinucleotide repeats, which result in clusters of repeated amino acids, frequently glycine and proline. Additionally, longer tandemly duplicated sequences are present as is a tetranucleotide sequence CCTG (CAGG) that was originally identified as a hotspot for homologous recombination [28] and was subsequently noted to occur at a high frequency near deletion breakpoints in human genes [29]. Mutational analyses of exon 1 revealed the occurrence of deletion/insertion mutations in repetitive regions of the exon (Table I and Fig. 1) [30,31]. Furthermore, all mutations occurred within 15 base pairs (bp) of the recombination/deletion hotspot sequence, CCTG; in four of the six mutations this sequence is present at deletion breakpoints. These data suggest that this region of the *WT1* gene is particularly susceptible to deletion/insertion mutations; nonsense and missense mutations have not been observed in exon 1 as they have been for other regions of the gene. Although this observation may, in part, be due to an inability to detect point mutations in this highly GC-rich exon and/or the lack of an effect on protein function of amino acid substitutions in this region, it appears that a particular mutational mechanism is important for the generation of mutations in exon 1.

Exon 1 deletion/insertion mutations are both somatic and germline, which suggests that, if the CCTG motif is playing a role, it acts both during mitosis and meiosis. Since two of the mutations occurred in tissue hemizygous for *WT1*, the mechanism by which the CCTG sequence may facilitate DNA deletions can involve a single DNA strand or sister chromatids. Interestingly, the one insertional mutation in exon 1 is also the one germline mutation observed. Furthermore, a disproportionate number of deletion/insertion mutations have been identified as somatic mutations in patients who are hemizygous at the *WT1* locus; seven such mutations are observed in tumors from nine hemizygous individuals (Table II). These data suggest that the genetic mechanism giving rise to these mutations may be enhanced by the hemizygous state.

As shown in a compilation of intragenic *WT1* mutations in WT patients (Table II), mutations have been detected throughout the gene. As discussed above, exon 1 mutations detected to date are all deletion/insertion mutations, the occurrence of which is probably due to the presence of tandem repeats and multiple copies of the recombination/deletion hotspot sequence, CCTG. However, the absence of identified single base changes in exon 1 is not likely to be due to such sequence motifs. Either point mutations are difficult to detect in this repetitive, GC-rich region, or amino acid substitutions in this part of the protein may generally have little effect on normal protein function and thus produce no aberrant phenotype.

As would be predicted by the two-hit mutational model for WT [32], the germline mutations shown in Table II almost invariably occur in patients with GU anomalies and/or bilateral disease. The two exceptions to this are females (Case 12 and S87-877), consistent with previous observations that females with germline *WT1* mutations often do not display a GU phenotype [30,33]. Furthermore, the presence of hemizygous somatic mutations in tumors from patients with large 11p13 germline deletions strongly supports the hypothesis that inactivation of both *WT1* alleles is important for tumorigenicity.

However, tumors have been described in which only a single heterozygous mutation has been detected. Assuming that the abolition of normal *WT1* protein function is requisite for tumorigenesis in those tumors associated with *WT1* mutations, this observation suggests that the aberrant protein encoded by the heterozygous mutation acts in a dominant negative manner. Data from a tumor, BM#7 (Table II), with compound heterozygous mutations are particularly relevant to the issue of whether truncated

TABLE I. Exon 1 Mutations in WT

Patient no.	Phenotype	Sex	Location NT*	Location Codon	Mutation	Type	Ref.
266672	WT	M	460	27	4 bp deletion	Somatic, heterozygous	31
186523	WT/GU	M	467	29	5 bp insertion	Germline, homozygous	31
802643	WT	F	583	68	4 bp deletion	Somatic, heterozygous	31
802649	WT/AN	F	610	77	34 bp deletion	Somatic, hemizygous	31
802501	WT/AN/GU	M	725	115	19 bp deletion	Somatic, hemizygous	31
S87-877	WT	F	748	123	5 bp deletion	Somatic, hemizygous	30

*NT = nucleotide numbering as per Ref. 39.

266672 (4bp deletion, codon 27)

GGCTGTGC**CCTG**C(**ctg**t)GAGCGGCGCGG

186523 (5bp insertion, codon 29)

TGTGC**CCTGCCTG**TGAG(GGGAG)CGGCGCGG

802643 (4bp deletion, codon 68)

CCGCCGCCGCCGC(ctca)CTCCTTCATCAAA**CAGG**

802649 (34bp deletion, codon 77)

GAGCCGAG(ctggggcggcgcggagccgcacgaggagcagtcc)**CTG**AGCGC

802501 (19bp deletion, codon 115)

TCCTCC(tccgcccagc**cagg**cgtca)TCCGGC**CAGGCCAGG**

S87-877 (5bp deletion, codon 123)

CAGGCGTCATCCG(gc**cag**)GC**CAGG**

Fig. 1. Nucleotide sequence of deletion/insertion mutations in *WT1* exon 1. Nucleotides deleted are in lower case in parentheses; nucleotides inserted are in large font in parentheses. Deletion hotspot consensus sequences CCTG (CAGG) are shown in boldface. Trinucleotide repeats are underlined.

WT1 proteins of varying length might act to block the function of wild-type protein. The two mutant alleles encode proteins truncated at codons in exon 3 and exon 8, respectively. The presence of two mutations implies that neither truncated protein acts as a dominant negative. These data suggest that similar truncation mutations, although perhaps observed as being heterozygous to a wild-type allele, do not act as dominant negative mutations.

Of the eight tumors from seven children carrying intragenic germline *WT1* mutations, seven are homozygous for the mutant allele (Table II). The one tumor (NP57) that remains heterozygous is from a bilateral WT patient whose contralateral tumor (NP58) is a homozygous mutant, which suggests that this mutation (encoding a protein truncated in the third zinc finger domain) acts recessively. If this is the case, an independent somatic mutation, as yet undetected, would be expected in tumor NP57. These data would suggest that the mutations act recessively. However, genetic (LOH) and epigenetic (loss of imprinting) alterations at chromosomal band *11p15, in particular* at the imprinted loci H19 and insulin-like growth factor II (IGF2), have been implicated in tumorigenesis [34–38]. Because of this, the observation in a tumor of 11p13 LOH and resultant homozygosity for a *WT1* mutation is difficult to interpret. Reduction of a *WT1* mutation to homozygosity may be necessary for tumorigenesis because the *WT1* mutation acts recessively or it may be a byproduct of genetic processes—somatic recombination or chromosome deletion or loss—whose functional significance is rendering 11p15 loci homozygous.

Data from two subsets of tumors presented here are relevant to this issue of the recessive vs. dominant action of *WT1* mutations. First, in those tumors reduced to homozygosity for germline *WT1* mutations (Table III), five of seven carry mutations encoding proteins truncated at codons in exons 1, 4, 6, and 9. As presented above, data from a tumor with compound heterozygous truncation mutations (BM#7) strongly imply that proteins truncated at codons in exons 3 and 8 do not act dominantly and suggest that the truncated proteins encoded by the germline mutations also do not have a dominant mode of action. In particular, the homozygous *WT1* mutation in tumor 186523 encodes a protein of only 29 of the normal amino acids, which is unlikely to have any normal or abnormal function. Thus, reduction of the *WT1* mutations to homozygosity in these tumors is likely important, although this does not imply that 11p15 LOH is unimportant in these tumors. It may well be that both 11p13 and 11p15 LOH, which are mediated through the same genetic mechanism, are critical steps in tumorigenesis.

The observation of hemizygous *WT1* mutations in tu-

TABLE II. Intragenic *WT1* Mutations in non-Drash WT Patients*

					Tumor status				
Patient no.	Phenotype	Sex	Exon	Mutation	Type	Mut	11p15	Effect on protein	Ref.
266672[a]	WT	M	1	4 bp del	Somatic	Het	n.d.	Frameshift/truncation	31
186523	WT/GU	M	1	5 bp ins	Germline	Hom	LOH	Frameshift/truncation	31
802643[a]	WT	F	1	4 bp del	Somatic	Het	RH	Frameshift/truncation	31
802649	WT/AN	F	1	34 bp del	Somatic	Hemi	RH	Frameshift/truncation	31
802501	WT/AN/GU	M	1	19 bp del	Somatic	Hemi	RH	Frameshift/truncation	31
S87-877	WT	F	1	5 bp del	n.d.	Hemi	RH	Frameshift/truncation	30
MW	WT	F	2	4 bp ins	Somatic	Het	n.d.	Frameshift/truncation	40
WT7	WT/AN/G/R	—	2 & 3	del	Somatic	Hemi	n.d.	Frameshift/truncation	41
12A	WT/other	M	2	5 bp del	Somatic	Hemi	RH	Frameshift/truncation	30
BT1	WT	M	2	C to T	n.d.	Het	n.d.	^{181}pro to ser	30
S86-169	bWT	F	5' 3	G to T	n.d.	Hom	NI	Splicing(?)/truncation	30
MR W		M	5' 3	1 bp ins	n.d.	Het	n.d.	Splicing(?)/truncation	30
PG	bWT/GU	M	4	17 bp del	Germline	Hom	n.d.	Frameshift/truncation	42
209942	bWT	F	6	1-2 kb del	Germline	Hom	LOH	Frameshift/truncation	33
TS	bWT/GU	M	6	G to T	Germline	Hom	n.d.	^{292}gly to val	42
DB	WT/GU	M	6	C to G	Germline	Hom	LOH	Splice site/truncation	43
IC	WT/AN/GU/R	F	3' 7	14 bp ins	Somatic	Hemi	n.d.	Splicing(?)/truncation	44
S86-1334	WT/AN/GU	F	7	1 bp del	Somatic	Hemi	RH	Frameshift/truncation	30
KK#33	WT	—	7	C to A	Somatic	n.d.	n.d.	^{338}ser to tyr	45
WT10	WT	—	8	C to T	Somatic	Het	n.d.	^{366}arg to cys	46
S87-52	WT	M	8	C to T	n.d.	Hemi	RH	^{372}his to tyr	30
BM#7	WT	—	8	C to T	Somatic	Het	n.d.	Nonsense/truncation	45
			3	7 bp ins	Somatic	Het	n.d.	Frameshift/truncation	45
MT#53	WT	—	8	C to T	Somatic	n.d.	n.d.	Nonsense/truncation	45
AH#20	WT	—	8	del/ins	Somatic	Het	n.d.	Frameshift/truncation	45
AR	WT	—	9	25 bp del	Somatic	Het	LOH	Frameshift/truncation	47
NP57[b]	bWT	—	9	C to T	Germline	Het	RH	Nonsense/truncation	46
NP58[b]	bWT	—	9	C to T	Germline	Hom	LOH	Nonsense/truncation	46
Case 12	WT	F	9	C to T	Germline	Hom	n.d.	^{394}arg to trp	48
WT 2A	WT/AN/GU	M	9	C to T	Somatic	Hemi	RH	Nonsense/truncation	30
DJ#11	WT	—	9	C to T	Somatic	Hom	n.d.	Nonsense/truncation	45
MF#88	WT	—	9	del/ins	Somatic	Hom	n.d.	Frameshift/truncation	45

*bWT = bilateral WT; R = mental retardation; Mut = mutation; del = deletion; ins = insertion; Het = heterozygous; Hom = homozygous; Hemi = hemizygous; RH = retention of heterozygosity; NI = not informative; n.d. = no data.

[a]*WT1* mutational analyses incomplete.

[b]Tumors from the same individual.

[c]Zygosity of *WT1* mutation and 11p15 loci in the tumor.

TABLE III. Tumors With Germline *WT1* Mutations in Non-Drash WT Patients*

			Tumor status				
Patient no.	Phenotype	Exon	Mutation	Mut	11p15	Effect on protein	Ref.
186523	WT/GU	1	5 bp ins	Hom	LOH	Frameshift/truncation	31
PG	bWT/GU	4	17 bp del	Hom	n.d.	Frameshift/truncation	42
209942	bWT	6	1-2 kp del	Hom	LOH	Frameshift/truncation	33
TS	bWT/GU	6	G to T	Hom	n.d.	^{292}gly to val	42
DB	WT/GU	6	C to G	Hom	LOH	Splice site/truncation	43
NP57[b]	bWT	9	C to T	Het	RH	Nonsense/truncation	46
NP58[b]	bWT	9	C to T	Hom	LOH	Nonsense/truncation	46
Case 12	WT	9	C to T	Hom	n.d.	^{394}arg to trp	48

*del = deletion; ins = insertion; Het = heterozygous; Hom = homozygous; RH = retention of heterozygosity; n.d. = no data.

[a]Zygosity of *WT1* mutation and 11p15 loci in the tumor.

[b]Tumors from the same individual.

TABLE IV. ***WT1*** **Germline Mutations in Drash Patients**

No. cases	Exon	Mutation	Effect on protein	Amino acid/ AA function[a]	Ref.
22	9	Missense	^{394}arg	DNA base contact	49,50,51
12	9	Missense	^{396}asp	DNA base contact	49,50,51,52
4	9	Intron	Splicing	Isoform skewing	49,53
2	8	Missense	^{366}arg	DNA base contact	49
2	8	Missense	^{373}his	DNA base contact	49,51
2	8	Missense	^{377}his	Zn interaction	49,50
1	8	Missense	^{355}cys	Zn interaction	52
1	8	Missense	^{366}cys	Zn interaction	51
1	8	Nonsense	^{362}arg/truncation		51
1	9	Missense	^{387}thr	?	49
1	7	Missense	cys	?	49
1	6	Missense	^{275}asp	?	49
1		11p13 deletion/reduced protein			49
1	9	1 bp ins	Frameshift/truncation		54
1	3	Nonsense	^{199}tyr/truncation		55
1	6	1 bp del	Frameshift/truncation		55

[a]AA = amino acid.

mors from patients with large germline deletions involving 11p13 (Table II) further argues that *WT1* haploinsufficiency, while apparently responsible for the development of GU anomalies, only predisposes to WT; complete inactivation of the gene is required for tumorigenicity. This again suggests that LOH at 11p13 is important in tumors. Interestingly, none of these tumors show LOH at 11p15. Although initially this could be interpreted as implying an uninvolvement of 11p15 genetic alterations in these tumors, mechanistic considerations may account for this observation. In these tumors 11p LOH would result in homozygous deletion of a large genomic region that might render the homozygous cells nonviable. Instead, more localized 11p15 events, such as 11p15-specific LOH or loss of imprinting of 11p15 genes, may occur in these cases.

Data from *WT1* mutational analyses of individuals with WT-associated Drash syndrome and WT patients with congenital GU anomalies in the absence of the Drash-characteristic renal mesangial sclerosis (WT/GU patients) have demonstrated gross differences between these two subsets of WT patients in the frequency of different types of mutations. In Drash patients a predominance of germline base substitutions that result in amino acid substitutions within the zinc finger domain at residues thought to be critical for binding of the protein to target DNA sequences are observed. Of the 54 cases presented in Table IV, 42 (78%) carry such missense mutations. In contrast, the identified germline 11p13 mutations in WT patients with congenital GU anomalies in the absence of Drash syndrome (WT/GU patients) are almost invariably either large deletions that encompass both the AN and *WT1* genes (as in WAGR syndrome individuals) or are small intragenic *WT1* deletions or point mutations that result in a truncated protein. For example, of those nine WT patients with GU anomalies listed in Table II, five carry germline 11p13 deletions and three carry mutations in exons 1, 4, and 6 that encode truncated proteins. Only one (TS) carries a germline missense mutation (in exon 6). Four of 54 (7%) Drash patients have sustained mutations that are predicted to alter the ratio of normally occurring splicing isoforms.

These data suggest that reduced levels of wild-type WT1 protein are responsible for the etiology of GU anomalies. In contrast, the presence of a single amino acid substitution, as is observed in the vast majority of Drash patients, alters the specificity or affinity of the aberrant protein for target DNA sequences, conferring a gain of function such that is now plays a critical role in the regulation of genes not usually appreciably regulated by *WT1*. An increased amount of a particular *WT1* isoform, as is suggested to occur in a few Drash patients, could have the same net affect as a protein with increased binding affinity. That different genetic modes of action—recessiveness, haploinsufficiency, and dominance—are involved in different phenotypes (WT, GU anomalies, and Drash-specific renal mesangial sclerosis) suggest that the *WT1* protein is critical for the transcriptional regulation of a number of genes, each of which is involved, individually, in the various phenotypes associated with *WT1* mutations.

However, notable exceptions that are not adequately explained by such a model have been described. Five of the 54 (9%) Drash patients listed in Table IV have sustained nonsense or deletion mutations predicted to result in grossly similar truncated proteins as those encoded by germline mutations in WT patients who do not display the Drash-characteristic renal pathology. And one patient (Case 12, Table II) has been described as carrying a

germline mutation that is the most commonly occurring mutation in Drash patients. This patient, a female, is described as having no evidence of renal failure at age 7.

The identification of genetic alterations such as chromosomal abnormalities, LOH, and gene mutations in WT patients and patients with WT-associated anomalies and correlation of those alterations with clinical phenotypes have resulted in the localization and isolation of at least one gene, *WT1*, that plays a role both in tumorigenesis and in developmental processes. That different types of *WT1* mutations with different genetic modes of action—recessiveness, haploinsufficiency, and dominance—are involved in different phenotypes (WT, GU anomalies, and Drash-specific renal mesangial sclerosis) suggests that the *WT1* protein is critical for the transcriptional regulation of a number of genes, each of which is involved in the various phenotypes associated with *WT1* mutations. Examination of genotype/phenotype correlations in patients continues to provide invaluable insight into the in vivo function of this gene. Further analysis of *WT1* mutations in patients and correlation of those mutations with clinical phenotypes, combined with in vitro *WT1* functional analyses, will ultimately result in the elucidation of the biological function of *WT1* in all its roles.

REFERENCES

1. Riccardi VM, Sujansky E, Smith AC, Francke U: Chromosomal imbalance in the aniridia-Wilms' tumor association: 11p interstitial deletion. Pediatrics 61:604–610, 1978.
2. Davis LM, Stallard R, Thomas GH, Couillin P, Junien C, Nowak NJ, Shows TB: Two anonymous DNA segments distinguish the Wilms' tumor and aniridia loci. Science 241:840–842, 1988.
3. Porteous DJ, Bickmore W, Christie S, Boyd PA, Cranston G, Fletcher JM, Gosden JR, Rout D, Seawright A, Simola KOJ, van Heyningen V, Hastie ND: HRAS1-selected chromosome transfer generates markers that colocalize aniridia- and genitourinary dysplasia-associated translocation breakpoints and the Wilms tumor gene within band 11p13. Proc Natl Acad Sci USA 84:5355–5359, 1987.
4. Breslow NE, Beckwith JB: Epidemiological features of Wilms' tumor: Results of the National Wilms' Tumor Study. J Natl Cancer Inst 68:429–436, 1982.
5. Geiser CF, Schindler AM: Long survival in a male with 18-trisomy syndrome and Wilms' tumor. Pediatrics 44:111–116, 1969.
6. Karayalcin G, Shanske A, Honigman R: Wilms' tumor in a 13-year-old girl with trisomy 18. Am J Dis Child 135:665–666, 1981.
7. Olson JM, Hamilton A, Breslow NE: Non-11p constitutional chromosome abnormalities in Wilms' tumor patients. Med Pediatr Oncol 24:305–309, 1995.
8. Hartley AL, Birch JM, Tricker K, Wallace SA, Kelsey AM, Harris M, Morris Jones PH: Wilms' tumour in the Li-Fraumeni cancer family syndrome. Cancer Genet Cytogenet 67:133–135, 1993.
9. Schimmenti LA, Cich JA, Gorlin RJ, Berry S: Autosomal dominant inheritance of Wilms' tumor and branchial cleft anomalies. Am J Hum Genet Suppl 53:502, 1993.
10. Kakinuma A, Morimoto I, Nakano Y, Fujimoto R, Ishida O, Okada Y, Inokuchi N, Fujihira T, Eto S: Familial primary hyperparathyroidism complicated with Wilms' tumor. Intern Med 33:123–126, 1994.
11. Szabo J, Heath B, Hill VM, Jackson CE, Zarbo RJ, Mallette LE, Chew SL, Besser GM, Thakker RV, Huff V, Leppert MF, Heath H: Hereditary hyperparathyroidism-Jaw tumor syndrome: The endocrine tumor gene HRPT2 maps to chromosome 1q21-q31. Am J Hum Genet 56:944–950, 1995.
12. Kondo K, Chilcote RR, Maurer HS, Rowley JD: Chromosome abnormalities in tumor cells from patients with sporadic Wilms' tumor. Cancer Res 44:5376–5381, 1984.
13. Slater RM: The cytogenetics of Wilms' tumor. Cancer Genet Cytogenet 19:37–41, 1986.
14. Douglass EC, Wilimas JA, Green AA, Look AT: Abnormalities of chromosomes 1 and 11 in Wilms' tumor. Cancer Genet Cytogenet 14:331–338, 1985.
15. Solis V, Pritchard J, Cowell JK: Cytogenetic changes in Wilms' tumors. Cancer Genet Cytogenet 34:223–234, 1988.
16. Kaneko Y, Homma C, Maseki N, Sakurai M, Hata J: Correlation of chromosome abnormalities with histological and clinical features in Wilms' and other childhood renal tumors. Cancer Res 51:5937–5942, 1991.
17. Wang-Wuu S, Soukup S, Bove K, Gotwals B, Lampkin B: Chromosome analysis of 31 Wilms' tumors. Cancer Res 50:2786–2793, 1990.
18. Maw M, Grundy PE, Millow LJ, Eccles MR, Dunn RS, Smith PJ, Feinberg AP, Law DJ, Paterson MC, Telzerow PE, Callen DF, Thompson AD, Richards RI, Reeve AE: A third Wilms' tumor locus on chromosome 16q. Cancer Res 52:3094–3098, 1992.
19. Grundy PE, Telzerow PE, Breslow N, Moksness J, Huff V, Paterson MC: Loss of heterozygosity for chromosomes 16q and 1p in Wilms' tumors predicts an adverse outcome. Cancer Res 54:2331–2333, 1994.
20. Malkin D, Sexsmith E, Yeger H, Williams BRG, Coppes MJ: Mutations of the p53 tumor suppressor gene occur infrequently in Wilms' tumor. Cancer Res 54:2077–2079, 1994.
21. Waber PG, Chen J, Nisen PD: Infrequency of ras, p53, WT1, or RB gene alterations in Wilms' tumors. Cancer 72:3732–3738, 1993.
22. Bardeesy N, Falkoff D, Petruzzi M-J, Nowak N, Zabel B, Adam M, Aguiar MC, Grundy P, Shows T, Pelletier J: Anaplastic Wilms' tumour, a subtype displaying poor prognosis, harbours p53 gene mutations. Nature Genet 7:91–97, 1994.
23. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, Rose EA, Kral A, Yeger H, Lewis WH, Jones C, Housman DE: Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 60:509–520, 1990.
24. Gessler M, Proustka A, Cavenee W, Neve RL, Orkin SH, Bruns GAP: Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. Nature 343:774–778, 1990.
25. Rauscher FJ III, Morris JF, Tournay OE, Cook DM, Curran T: Binding of the Wilms' tumor locus zinc finger protein to the EGR-1 consensus sequence. Science 250:1259–1262, 1990.
26. Madden SL, Cook DM, Morris JF, Gashler A, Sukhatme VP, Rauscher FJ III: Transcriptional repression mediated by the *WT1* Wilms' tumor gene product. Science 253:1550–1553, 1991.
27. Wang Z-Y, Qui Q-Q, Deuel TF: The Wilms' tumor gene product WT1 activates or suppresses transcription through separate functional domains. J Biol Chem 268:9172–9175, 1993.
28. Steinmetz M, Uematsu Y, Lindahl KF: Hotspots of homologous recombination in mammalian genomes. Trends Genet 3:7–10, 1987.
29. Krawczak M, Cooper DN: Gene deletions causing human genetic disease: Mechanisms of mutagenesis and the role of the local DNA sequence environment. Hum Genet 86:425–441, 1991.
30. Gessler M, Konig A, Arden K, Grundy P, Orkin S, Sallan S, Peters C, Ruyle S, Mandell J, Li F, Cavenee W, Bruns G: Infrequent mutation of the WT1 gene in 77 Wilms' tumors. Hum Mutat 3:212–222, 1994.

31. Huff V, Jaffe N. Saunders GF, Strong LC, Villalba F, Ruteshouser EC: WT1 exon 1 deletion/insertion mutations in Wilms' tumor patients associated with di- and trinucleotide repeats and deletion hotspot consensus sequences. Am J Hum Genet 56:84–90, 1995.
32. Knudson AG, Strong LC: Mutation and cancer: A model for Wilms' tumor of the kidney. J Natl Cancer Inst 48:313–324, 1972.
33. Huff V, Miwa H, Haber DA, Call KM, Housman D, Strong LC, Saunders GF: Evidence for *WT1* as a Wilms tumor (WT) gene: Intragenic germinal deletion in bilateral WT. Am J Hum Genet 48:997–1003, 1991.
34. Mannens M, Slater RM, Heyting C, Bliek J, de Kraker J, Coad N, de Pagter-Holthuizen P, Pearson PL: Molecular nature of genetic changes resulting in loss of heterozygosity of chromosome 11 in Wilms' tumours. Hum Genet 81:41–48, 1988.
35. Reeve AE, Sih SA, Raizis AM, Feinberg AP: Loss of allelic heterozygosity at a second locus on chromosome 11 in sporadic Wilms' tumor cells. Mol Cell Biol 9:1799–1803, 1989.
36. Wadey RB, Pal N, Buckle B, Yeomans E, Pritchard J, Cowell JK: Loss of heterozygosity in Wilms' tumour involves two distinct regions of chromosome 11. Oncogene 5:901–907, 1990.
37. Moulton T, Crenshaw T, Hao Y, Moosikasawan J, Lin N, Dembitzer F, Hensle T, Weiss L, McMorrow L, Loew T, Kraus W, Gerald W, Tycko B: Epigenetic lesions at the H19 locus in Wilms' tumour patients. Nature Genet 7:440–447, 1994.
38. Steenman MJC, Rainier S, Dobry CJ, Grundy P, Horon IL, Feinberg AP: Loss of imprinting of IGF2 is linked to reduced expression and abnormal methylation of H19 in Wilms' tumour. Nature Genet 7:433–439, 1994.
39. Gessler M, Konig A, Bruns GAP: The genomic organization and expression of the *WT1* gene. Genomics 12:807–813, 1992.
40. Park S, Bernard A, Bove KE, Sens DA, Hazen-Martin DJ, Garvin AJ, Haber DA: Inactivation of *WT1* in nephrogenic rests, genetic precursors to Wilms' tumour. Nature Genet 5:363–367, 1993.
41. Brown KW, Wilmore HP, Watson JE, Mott MG, Berry PJ, Maitland NJ: Low frequency of mutations in the *WT1* coding region in Wilms' tumor. Genes Chromosome Cancer 8:74–79, 1993.
42. Pelletier J, Bruening W, Li FP, Haber DA, Glaser T, Housman DE: *WT1* mutations contribute to abnormal genital system development and hereditary Wilms' tumour. Nature 353:431–434, 1991.
43. Schneider S, Wildhardt G, Ludwig R, Royer-Pokora B: Exon skipping due to a mutation in a donor splice site in the *WT-1* gene is associated with Wilms' tumor and severe genital malformations. Hum Genet 91:599–604, 1993.
44. Santos A, Osorio-Almeida L, Baird PN, Silva JM, Boavida MG, Cowell J: Insertional inactivation of the *WT1* gene in tumour cells from a patient with WAGR syndrome. Hum Genet 92:83–86, 1993.
45. Veranasi R, Bardeesy N, Ghahremani M, Petruzzi M-J, Nowak N, Adam MA, Grundy P, Shows TB, Pelletier J: Fine structure analysis of the WT1 gene in sporadic Wilms tumors. Proc Natl Acad Sci USA 91:3554–3558, 1994.
46. Little MH, Prosser J, Condie A, Smith PJ, Van Heyningen V, Hastie ND: Zinc finger point mutations within the *WT1* gene in Wilms tumor patients. Proc Natl Acad Sci USA 89:4791–4795, 1992.
47. Haber DA, Buckler AJ, Glaser T, Call KM, Pelletier J, Sohn RL, Douglass EC, Housman DE: An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor. Cell 61:1257–1269, 1990.
48. Akasaka Y, Kikuchi H, Nagai T, Hiraoka N, Kato S, Hata J: A point mutation found in the WT1 gene in a sporadic Wilms' tumor without genitourinary abnormalities is identical with the most frequent point mutation in Denys-Drash syndrome. FEBS Lett 317:39–43, 1993.
49. Coppes MJ, Huff V, Pelletier J: Denys-Drash syndrome: Relating a clinical disorder to genetic alterations in the tumor suppressor gene *WT1*. J Pediatr 123:673–678, 1993.
50. Nordenskjold A, Friedman E, Anvret M: *WT1* mutations in patients with Denys-Drash syndrome: A novel mutation in exon 8 and paternal allele origin. Hum Genet 93:115–120, 1994.
51. Little MH, Williamson KA, Mannens M, Kelsey A, Gosden C, Hastie ND, van Heyningen V: Evidence that *WT1* mutations in Denys-Drash syndrome patients may act in a dominant-negative fashion. Hum Mol Genet 2:259–264, 1993.
52. Sakai A, Tadokoro K, Yanagisawa H, Nagafuchi S, Hoshikawa N, Suzuki T, Kohsaka T, Hasegawa T, Nakahori Y, Yamada M: A novel mutation of the *WT1* gene (a tumor supressor gene for Wilms' tumor) in a patient with Denys-Drash syndrome. Hum Mol Genet 2:1969–1970, 1993.
53. Konig A, Jakubiczka S, Wieacker P, Schlosser HW, Gessler M: Further evidence that imbalance of *WT1* isoforms may be involved in Denys-Drash syndrome. Hum Mol Genet 2:1967–1968, 1993.
54. Ogawa O, Eccles MR, Yun K, Mueller RF, Holdaway MDD, Reeve AE: A novel insertional mutation at the third zinc finger coding region of the *WT1* gene in Denys-Drash syndrome. Hum Mol Genet 2:203–204, 1993.
55. Bardeesy N, Zabel B, Schmitt K, Pelletier J: *WT1* mutations associated with incomplete Denys-Drash syndrome define a domain predicted to behave in a dominant-negative fashion. Genomics 21:663–665, 1994.

COMMENTARY

Huff provides a complete overview of the correlation between the different genetic alterations, especially those found in *WT1* (Tables I–IV), and the resulting clinical phenotypes. For example, WT patients with constitutional *WT1* mutations virtually all have either bilateral disease and/or GU malformations (Table II). The exception to this are females in whom constitutional *WT1* mutations do not result in GU malformations. This is likely due to the fact that mammals are genetically programmed for female development and male development requires a cascade of events encoded by the Y chromosome. Earlier, it was postulated that the *WT1* protein might only affect the genetic events required for male development by interfering with the signals encoded by the Y chromosome [1]. This hypothesis would also explain why most patients with the Denys-Drash syndrome who have a female karyotype (46,XX) have normal female external genitalia, while virtually all with a male karyotype (46,XY) have ambiguous genitalia (Table IV). Finally, of particular interest in this presentation are the mutations described in *WT1* exon 1 (Table I). Until recently, no mutations had been described in this region, which is very difficult to analyze [2].

REFERENCES

1. Coppes MJ, Huff V, Pelletier J: Denys-Drash syndrome: Relating a clinical disorder to genetic alterations in the tumor suppressor gene *WT1*. J Pediatr 123:673–678, 1993.
2. Coppes MJ, Campbell CE, Williams BRG: "Wilms' Tumor: Clinical and Molecular Characterization." Austin, TX: RG Landes Company, 1995.